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Postprandial Interleukin-6 Release from Skeletal Muscle in Men with Impaired Glucose Tolerance Can Be Reduced by Weight Loss

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Context: Obesity and type 2 diabetes mellitus are associated with increased levels of IL-6, a marker of inflammation.

Objective: This study addressed the question of whether IL-6 was released from skeletal muscle after a high-fat meal in men with impaired glucose tolerance (IGT), a prediabetic state, and whether IL-6 release could be reduced by weight loss.

Design: Skeletal muscle metabolism was studied in men with IGT ($n = 11$) and compared with men with normal glucose tolerance (NGT, $n = 9$), matched for body mass index and age. IL-6 flux over skeletal muscle was measured with the forearm model. Eight IGT men were willing to participate in a 12-wk weight loss program and were tested again.

Results: IL-6, but not C-reactive protein or TNF- α receptor 1 and 2, was released by skeletal muscle. Muscle IL-6 release was higher in IGT than in NGT during fasting (IGT = 2.26 ± 1.89 vs. NGT = 0.87 ± 0.48 fmol*100 ml tissue $^{-1}$ *min $^{-1}$, $P = 0.04$) and after a meal (mean area under the curve per minute: IGT = 3.48 ± 2.63 vs. NGT = 1.37 ± 0.75 fmol*100 ml tissue $^{-1}$ *min $^{-1}$, $P = 0.03$). In the IGT men, body weight loss resulted in a decrease of postprandial IL-6 release from skeletal muscle (-52% ; $P = 0.04$), reaching levels of the obese, NGT controls.

Conclusion: The present data suggest that a high-fat meal can evoke IL-6 release from muscle and that the IL-6 release is a consequence rather than a cause of the obese, insulin-resistant, and/or IGT state. (*J Clin Endocrinol Metab* 90: 5819–5824, 2005)

OBESITY AND TYPE 2 diabetes mellitus (DM2) have been associated with subclinical inflammation. Markers of inflammation, like the cytokine IL-6 and the acute-phase protein C-reactive protein (CRP), are elevated in obese, insulin-resistant subjects (1–3). Also TNF- α receptors, as markers for TNF- α action (4), and to a lesser extent TNF- α itself, have been found to be elevated in patients with DM2 (3) and, in some studies, also in subjects with impaired glucose tolerance (IGT), a prediabetic state (5). IL-6 is a cytokine that is produced by many cell types and is increased in cancer cachexia (6), after endotoxin stimulation (7) and after vaccination (8). IL-6 is also increased in obesity, insulin resistance, and DM2, and plasma IL-6 has been shown to be predictive of the development of DM2 in several prospective studies (9, 10). So, besides its role in inflammation, IL-6 may also affect insulin sensitivity or glucose metabolism.

It was suggested that IL-6 may cause insulin resistance in organs like liver and skeletal muscle. In human HepG2 cells

and primary mouse hepatocytes, IL-6 has been shown to impair insulin signaling by reducing the tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and reducing the association of the p85 subunit of phosphatidylinositol 3-kinase with IRS-1 (11). Insulin receptor autophosphorylation and IRS-1 tyrosine phosphorylation were also reduced *in vivo* in the livers of mice after chronic infusion with IL-6 (12). On the other hand, in the same study, no effect was found on skeletal muscle. In addition, IL-6 administration to myocytes in culture had no effect on insulin action (13) and a short-term infusion of IL-6 in rats did not affect insulin sensitivity or whole-body glucose uptake (14). A study with healthy young men showed that the infusion of high amounts of recombinant human IL-6 did not alter glucose uptake or oxidation. This shows that acute high concentrations of IL-6 might not disturb insulin sensitivity or glucose disposal in skeletal muscle in healthy subjects (15).

The observation that increased IL-6 concentrations are associated with DM2 leads to the suggestion that the systemic production of IL-6 is increased in the insulin-resistant state. It is known that adiposity plays a role in subclinical inflammation, because adipose tissue produces inflammation markers like IL-6 (16). Recently however, the skeletal muscle has become an interesting organ in the production of IL-6 as well, because it released IL-6 during and after exercise (17, 18). So far, few studies addressed the role of muscle IL-6 production in man in the development of insulin resistance

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Abbreviations: AUC, Area under the curve; BMI, body mass index; CRP, C-reactive protein; CRP-s, soluble CRP; CV, coefficient of variation; DM2, type 2 diabetes mellitus; E%, energy percent; FFM, fat-free mass; IGT, impaired glucose tolerance; IRS, insulin receptor substrate; NGT, normal glucose tolerance; WL, weight loss.

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in conditions like IGT and DM2. During exercise, no significant differences were found in muscle IL-6 production in skeletal muscle between controls and DM2 patients (19). However, an important stressor next to exercise is the intake of a high-fat meal. In particular in DM2, the impaired handling of substrates in the postprandial phase may be of importance in the development or deterioration of insulin resistance and hyperglycemia. It has been found before that venous IL-6 concentrations increased after a high-fat meal in obese men (20). Additionally, it has been shown that after a high-fat meal, the venous IL-6 concentrations slightly increased in healthy overweight subjects, and increased even more in newly diagnosed diabetic subjects (21).

The aim of this study was to examine 1) whether skeletal muscle produces IL-6 during fasting or in the postprandial phase; 2) whether the production of IL-6 by skeletal muscle is increased in obese men with IGT compared with obese men with NGT; and if so, 3) whether the production of IL-6 in skeletal muscle changes after weight loss in IGT men. In parallel with IL-6, other inflammatory markers like CRP and TNF- α receptors were monitored.

Subjects and Methods

Subjects

Eleven obese men with IGT and nine obese men with normal glucose tolerance (NGT) matched for age and body mass index (BMI) participated in the study. Inclusion criteria were obesity (BMI > 30 kg/m²), diastolic blood pressure less than 100 mm Hg, no major health problems, and no use of medication that could influence the measurements. The IGT men had no family history of diabetes. Subjects were not involved in any regular physical activity for more than 3 h a week. The participants were asked not to change their physical activity and smoking habits during the study. One IGT person smoked. The experimental protocol was approved by the local Medical Ethical Committee of the Maastricht University. All subjects gave written informed consent.

Glucose tolerance, body composition, aerobic capacity, and insulin sensitivity

Subjects were screened for glucose metabolism with a standard oral glucose tolerance test (75 g glucose) with capillary blood sampling at baseline and after 2 h. Subjects were included according to the World Health Organization criteria of 1999 for capillary plasma (IGT: fasting <7.0 mmol/liter, 2 h postload >8.9 and <12.2 mmol/liter). Two subjects with glucose values (fasting <8.0 mmol/liter and 2 h postload <14.8 mmol/liter) above the cutoff points were included as well. After inclusion, fat mass and fat-free mass (FFM) were determined by underwater weighing with correction for residual lung volume (Volugraph 2000; Mijnhardt, Bunnik, The Netherlands) and calculated according to Siri (22). Extracellular water was estimated using bio-impedance (Hydra; Xitron Utilities, San Diego, CA). Maximal aerobic power (W_{max}) and peak oxygen uptake (VO_{2peak}) were determined during an incremental exhaustive exercise test on an electronically braked bicycle ergometer (Lode, Groningen, The Netherlands). Insulin sensitivity was measured with a hyperinsulinemic euglycemic clamp (23). Insulin (1 mU/kg BW⁻¹·min⁻¹) was infused at a constant rate with glucose clamped at 4.6 mmol/liter with an iv infusion of 20% wt/vol glucose infusion. M value was calculated as the glucose infusion rate (millimoles of glucose per minute) per kilogram FFM during a steady-state of 30 min after at least 120 min of insulin infusion.

High-fat meal test

Subjects were informed to refrain from heavy exercise like organized sports activities or heavy physical work and to consume a carbohydrate-rich dinner like pasta the day before the test. Subjects came to the laboratory by car or public transport after an overnight fast (12–14 h),

during which they were only allowed to drink water. Forearm skeletal muscle was studied with the forearm model by measuring arterio-venous concentration differences across the muscle in combination with forearm blood flow measurements (24). Two catheters were inserted, one in the arteria radialis and one in a retrograde direction into a deep forearm vein. The O₂-saturation in the deep venous forearm samples was less than 60%, implying that deep venous blood was drawn mainly from skeletal muscle. Blood sampling and blood flow measurements took place before and after a high-fat meal. The meal provided 2.6 MJ, which consisted of 61 energy percent (E%) from fat (of which 35.5 E% saturated, 18.8 E% monounsaturated, and 1.7 E% polyunsaturated fat), 33 E% carbohydrates and 6 E% protein. To exclude metabolism in the hand, the hand circulation on the side of the deep venous catheter was occluded (>200 mm Hg) at every measurement. Immediately after occlusion, blood flow was measured with venous occlusion plethysmography (EC5R plethysmograph; Hokanson, Bellevue, WA). One minute after occlusion, arterial and deep venous blood samples were simultaneously drawn in EDTA syringes (1.5 mg EDTA/ml) for plasma analyses before (at –60, –30, and 0 min) and 30, 60, 90, 120, 180, and 240 min after the test meal. Blood samples were immediately centrifuged and plasma was frozen in liquid nitrogen and stored for analysis at –80 C. Plasma flow was calculated as bloodflow*[1 – (hematocrit/100)]. Metabolite fluxes were calculated as arterio-venous differences of metabolites multiplied by plasma flow. A positive flux is uptake from plasma and a negative flux is release by muscle.

Weight loss period

After the measurements, eight men from the IGT group were willing to follow a 12-wk weight loss program and visited a dietitian every week. Every week the subjects were weighed and capillary plasma glucose was checked. During the first 4 wk, the subjects were provided with a very low calorie diet (2 MJ/d) based on shakes (Modifast; Nutrition et Santé, Breda, The Netherlands) containing all the essential macro- and micro-nutrients. Beside the shakes, subjects were stimulated to eat at least 150 g of raw nonstarchy vegetables each day. From wk 5–8, the shakes were gradually replaced by meals, increasing the energy content of the diet up to 4.2 MJ/d. In wk 8–12, the subjects were kept in energy balance by prescribing detailed weekly menus. All measurements, *i.e.* clamp, forearm model, hydrostatic weighing, and the exercise test, were repeated in wk 11 and 12.

Biochemical analysis

Plasma free fatty acids and glucose were analyzed using standard enzymatic techniques automated on the COBAS Fara centrifugal analyzer at 340 nm (for FFA: FFA-C test kit, Wako Chemicals, Neuss, Germany; for glucose: Roche Unikit III, Hoffman-la-Roche, Basel, Switzerland). Insulin was analyzed using a fluorimetric assay (autoDELFI Insulin; PerkinElmer, Turku, Finland) with no cross-reactivity with proinsulin or split forms of proinsulin. IL-6 was analyzed with an ultra-sensitive ELISA kit (Quantikine HS SixPak; R&D Systems, Abingdon, UK; intraassay coefficient of variation (CV) of 3.8%), Soluble CRP (CRP-s) was analyzed on an autoanalyzer Hitachi 912 (High Sensitive kit 11972855; Roche, Almere, The Netherlands; intraassay CV of 2.6%). The TNF receptors R1 and R2 (soluble) were analyzed with an ELISA kit from Biosource (TNF-R1/p55: KAC1762, intraassay CV of 7.9%; TNF-R2/p75: KAC1772, intraassay CV of 6.6%; Nivelles, Belgium). All metabolites were analyzed in duplicate (duplicate CV < 10%).

Statistical analysis

NGT and IGT men were compared with a two-tailed Student's *t* test for independent samples. IGT men before and after weight loss were compared with a two-tailed Student's *t* test for paired samples. For fasting values the average of time points –60, –30, and 0 min was taken. For postprandial responses the total areas under the curve (AUC) divided by time (AUC/min) were compared. Results in the tables and text are given in mean \pm SD. Data in the figures are given in mean \pm SEM. A *P* value less than 0.05 was considered as statistically significant. Statistical analysis was performed using SPSS 10.0 for Macintosh.

Results

General

No differences were seen in age, BMI, body fat percentage, and aerobic capacity between the IGT men and the NGT men. Insulin sensitivity was not significantly different (Table 1).

Fasting metabolites and postprandial response in forearm muscle

Fasting concentrations of glucose, insulin, and free fatty acids were not different between IGT and NGT men. Postprandially, arterial glucose tended to increase more ($P = 0.10$) in the IGT men, but the postprandial increase of arterial insulin and the postprandial decrease of arterial free fatty acid concentrations were not different between NGT and IGT (Table 2). Arterial IL-6 concentrations were not different between groups in the fasting state, nor after the meal. However, deep venous IL-6 concentrations were higher in the IGT men during fasting (IGT = 3.89 ± 1.99 vs. NGT = 2.31 ± 0.73 pg/ml, $P = 0.03$) and in the postprandial phase (AUC/min: IGT = 5.06 ± 3.18 vs. NGT = 2.86 ± 0.77 pg/ml, $P = 0.05$; Fig. 1). Similar differences were found for IL-6 flux, because blood flow was not different between groups during fasting (NGT = 1.51 ± 0.47 ; IGT = 1.78 ± 0.60 ml*100 ml tissue⁻¹*min⁻¹) or after a meal (AUC/min, NGT = 1.82 ± 0.62 ; IGT = 1.97 ± 0.85 ml*100 ml tissue⁻¹*min⁻¹). It was found that the IL-6 release from muscle was higher in the IGT group than in the NGT group, both in the fasting state (IGT = 2.26 ± 1.89 vs. NGT = 0.87 ± 0.48 fmol*100 ml tissue⁻¹*min⁻¹, $P = 0.04$) and in the postprandial phase (AUC/min: IGT = 3.48 ± 2.63 vs. NGT = 1.37 ± 0.75 fmol*100 ml tissue⁻¹*min⁻¹, $P = 0.03$; Fig. 2). The postprandial IL-6 release (AUC/min) was higher than the fasting IL-6 release ($P < 0.05$) in the group as a whole. Arterial CRP, TNF-R1, and TNF-R2 concentrations were not different between groups (Table 3). CRP, TNF-R1, and TNF-R2 arteriovenous differences were all less than 1% of arterial concentrations. Subsequently all fluxes were around zero, showing that CRP, TNF-R1, and TNF-R2 were not released from muscle, neither in the fasting nor in the postprandial phase.

Extrapolated estimated muscle IL-6 production

It can be calculated to what extent the release of IL-6 from muscle can contribute to systemic IL-6 levels. It has to be taken into account that these are calculated estimates, partly based on assumptions. We assumed that muscle mass, estimated as approximately 55% (25) of FFM, released IL-6 at the same rate as the forearm muscle studied here, and used an estimated half-life of 3 min (26). We estimated the IL-6 production rate of total muscle mass during fasting (mean_{TOTAL GROUP} = 0.58 ng/min, range 0.12–2.11 ng/min) and postprandially (mean_{TOTAL GROUP} = 0.90 ng/min, range 0.06–2.61 ng/min). The estimated contribution of IL-6 release from skeletal muscle to systemic IL-6, using whole body extracellular fluid estimated from bio-impedance (Hydra; Xitron Utilities), was 12% (range 2–42%) for the whole group in the fasting state. In the postprandial phase it was 11% (range 2–21%) in NGT men and highest in IGT men (25%, range 2–66%).

Effect of weight loss

Eight IGT men were willing to participate in the weight loss program. They lost on average 15.0 ± 3.4 kg of body weight (Table 1). Approximately 72% of this weight loss was owing to a reduced fat mass and 28% to a reduction in FFM. Body weight was 82.4 ± 8.3 kg in wk 8, at the end of the weight loss period, and 82.5 ± 9.4 kg in wk 10, just before repeating the measurements, indicating that subjects were in energy balance during the measurement period. Weight loss improved insulin sensitivity (Table 1), reduced fasting arterial glucose, insulin, and free fatty acid concentrations and reduced postprandial hyperglycemia (–8%) and hyperinsulinemia (–41%) (Table 2). No change was seen in aerobic capacity (Table 1).

After weight loss (WL), no significant change was observed in fasting arterial IL-6 concentrations (Table 3) or fasting IL-6 release from muscle (IGT_{PRE WL}: 2.22 ± 1.86 vs. IGT_{AFTER WL}: 1.40 ± 1.32 fmol*100 ml tissue⁻¹*min⁻¹; Fig. 1; $P = 0.24$), but postprandial IL-6 release had decreased (IGT_{PRE WL}: 2.99 ± 2.58 vs. IGT_{AFTER WL}: 1.45 ± 1.33 fmol*100 ml tissue⁻¹*min⁻¹, $P = 0.04$) after weight loss (Fig. 2). Weight

TABLE 1. General and metabolic characteristics of the obese men

	NGT (n = 9)	IGT (n = 11)	IGT	
			Before WL (n = 8)	After WL (n = 8)
Age (yr)	55.9 ± 7.2	55.5 ± 7.9	56.1 ± 8.4	
Body weight (kg)	107.0 ± 19.2	95.9 ± 7.8	95.2 ± 7.6	80.2 ± 7.6 ^a
FFM (kg)	69.5 ± 10.8	64.8 ± 5.0	63.2 ± 3.6	58.9 ± 4.2 ^a
BMI (kg/m ²)	34.6 ± 4.8	32.1 ± 2.3	32.2 ± 4.4	27.5 ± 1.5 ^a
Body fat %	34.6 ± 6.0	32.5 ± 3.0	33.6 ± 2.1	26.3 ± 3.6 ^a
Waist to hip ratio	1.02 ± 0.05	1.03 ± 0.04	1.04 ± 0.03	0.99 ± 0.03 ^a
VO ₂ peak (ml O ₂ *kg FFM ⁻¹ *min ⁻¹)	39.7 ± 6.0	41.0 ± 4.8	39.7 ± 4.3	40.9 ± 7.2
Glucose, fasting (capillary, mmol/liter)	5.7 ± 0.6	6.5 ± 0.9 ^b	6.7 ± 1.0	n.d.
Glucose, 2 h after 75-g glucose load (capillary, mmol/liter) (mg/dl)	6.7 ± 1.2	11.9 ± 2.0 ^c	12.7 ± 2.0	n.d.
Insulin sensitivity (M value, μmol*kg FFM ⁻¹ *min ⁻¹)	32.4 ± 14.3	24.5 ± 10.8	23.3 ± 9.4	39.2 ± 11.7 ^a

n.d., Not determined.

^a $P < 0.01$ IGT before weight loss vs. after weight loss; paired test with n = 8.

^b $P < 0.05$ NGT vs. IGT.

^c $P < 0.01$ NGT vs. IGT.

TABLE 2. Fasting and postprandial arterial concentrations of glucose, insulin, and free fatty acids in men with NGT, IGT, and IGT after weight loss

	NGT (n = 9)	IGT (n = 11)	IGT	
			Before WL (n = 8)	After WL (n = 8)
Glucose, fasting (mmol/liter)	5.8 ± 0.3	6.0 ± 0.5	5.9 ± 0.6	5.3 ± 0.5 ^a
Postprandial AUC	6.57 ± 0.47	7.08 ± 0.65 ^b	7.07 ± 0.75	6.47 ± 0.33 ^a
Insulin, fasting (mU/liter)	18.0 ± 12.7	14.4 ± 3.8	14.3 ± 4.5	6.5 ± 3.1 ^c
Postprandial AUC	31.5 ± 13.7	38.2 ± 14.7	34.5 ± 11.5	20.9 ± 9.0 ^a
Free fatty acid, fasting (μmol/liter)	555 ± 166	675 ± 184	697 ± 201	556 ± 102 ^a
Postprandial AUC	359 ± 70	440 ± 148	473 ± 163	360 ± 92 ^a

Mean ± SD. Postprandial AUC as mean per minute (AUC/min).

^a $P < 0.05$ IGT before weight loss *vs.* after weight loss; paired test with $n = 8$.

^b $P < 0.10$ difference between NGT and IGT.

^c $P < 0.01$ IGT before weight loss *vs.* after weight loss; paired test with $n = 8$.

loss also decreased the arterial CRP level (−45%, $P = 0.004$, Table 3), whereas the TNF-R1 receptor showed a trend for a lower concentration (−7%, $P = 0.06$, Table 3). No correlations between M value, body weight loss, and IL-6 release were found.

Discussion

A major finding of the present study is that the cytokine IL-6 was released from skeletal muscle of obese IGT and obese NGT men during fasting and after a high-fat meal. Interestingly, the IL-6 release from forearm muscle was higher in obese men with IGT compared with obese men with NGT. Furthermore, in IGT men, the IL-6 release from muscle after a high-fat meal was reduced after weight loss, in concert with improvements in insulin sensitivity and glucose tolerance.

It has been reported before that the venous IL-6 concentration increases after a meal, especially after a high-fat meal (20, 21). This is the first report describing IL-6 release in the postprandial phase by skeletal muscle. In the present study, it can be calculated that the estimated total muscle mass would produce 7 *vs.* 16% of the systemic IL-6 during fasting and 11 *vs.* 25% in the postprandial phase for NGT and IGT subjects, respectively. Mohamed-Ali *et al.* (27) previously

reported that the estimated production rate of whole body adipose tissue was 1.41 ng/min (range 0.22–2.67 ng/min), which was in the same range as the values we found for muscle in the postprandial phase in IGT men before weight loss (1.17 ng/min, range 0.15–2.61 ng/min). Thus, both adipose tissue and skeletal muscle contribute to circulating IL-6 concentrations. The question remains whether the IL-6 is produced by myocytes or by other cell types present in muscle, because it has recently been found that the IL-6 release from adipose tissue derives 90% from nonadipocytes (28). Although it is known that IL-6 can be released by muscle fibers themselves (13, 29), the contribution of other cell types such as monocytes/macrophages, endothelial cells, fibroblasts, and smooth muscle cells cannot be excluded. The contribution of other tissues in the forearm, like adipose tissue, cannot be entirely excluded either, although the deep venous plasma was mainly drawn from muscle, as confirmed by an O_2 -saturation less than 60%.

It is still unclear what may trigger IL-6 release from muscle. We cannot even exclude that the experimental conditions *per se* may have had an effect on IL-6 release from muscle, because we did not include a control group for the meal-effect. Nevertheless, it is likely that the meal *per se* evoked an

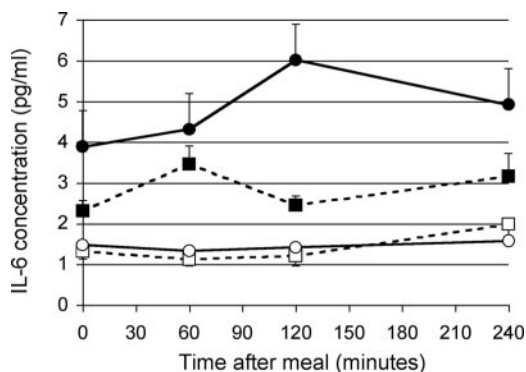


FIG. 1. Arterial and deep venous concentrations of IL-6 across forearm muscle during fasting and after a meal; compare NGT and IGT men. Arterial IGT (○), deep venous IGT (●), arterial NGT (□), deep venous NGT (■). Means ± SEM. Groups were compared with an unpaired Student's *t* test: arterial IL-6 concentration during fasting, $P = 0.492$; deep venous IL-6 concentration, $P = 0.031$; postprandial AUC for arterial IL-6 concentration, $P = 0.698$; postprandial AUC for deep venous IL-6 concentration, $P = 0.049$. Arterial *vs.* venous concentrations, paired Student's *t* test: all differences $P < 0.01$.

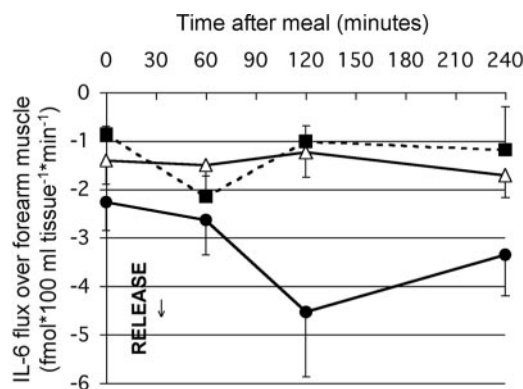


FIG. 2. IL-6 flux over skeletal muscle during fasting and after a meal; compare obese NGT men (obese NGT, $n = 9$) with obese men with IGT before (obese IGT, $n = 11$) and after weight loss (IGT after weight loss, $n = 8$). NGT men (■), IGT men before weight loss (●), IGT men after weight loss (△). Means ± SEM. NGT men and IGT men before weight loss were compared with a Student's *t* test for independent samples: IL-6 flux during fasting, $P = 0.038$; postprandial AUC for IL-6 flux, $P = 0.026$. IGT men ($n = 8$) before and after weight loss were compared with a Student's *t* test for paired samples: IL-6 flux during fasting, $P = 0.301$; postprandial AUC for IL-6 flux, $P = 0.042$.

TABLE 3. Fasting arterial concentrations of IL-6, CRP, and TNF- α receptors in men with NGT, IGT, and IGT after weight loss

	NGT (n = 9)	IGT (n = 11)	P value	IGT before WL (n = 8)	IGT after WL (n = 8)	P value
IL-6	1.33 \pm 0.57	1.47 \pm 0.42	0.57	1.58 \pm 0.40	1.41 \pm 0.41	0.23
CRP-s	2.21 \pm 1.71	3.46 \pm 1.90	0.15	4.21 \pm 1.75	2.09 \pm 1.15	0.004
TNF-R1	1.52 \pm 0.22	1.45 \pm 0.23	0.55	1.45 \pm 0.27	1.35 \pm 0.28	0.06
TNF-R2	3.27 \pm 0.89	3.65 \pm 0.87	0.42	3.59 \pm 0.67	3.65 \pm 2.01	0.79

Mean \pm SD. IL-6 is given in picograms per milliliter, CRP-s in milligrams per liter, and TNF-R1 and TNF-R2 are given in nanograms per milliliter.

effect on IL-6 release, because the postprandial IL-6 release (AUC/min) was higher than the fasting IL-6 release ($P < 0.05$) in the group as a whole. Several factors may provoke IL-6 release from muscle. In the first place, the production of cytokines in skeletal muscle, like IL-6, may be stimulated by oxidative stress (30). One of the factors that may cause oxidative stress in the postprandial period is impaired substrate handling (31), as described in IGT and DM2 (24, 32). Impairments in lipid (33) and glucose (34) handling may both lead to oxidative stress. In the second place, impairments in lipid metabolism, like a reduced oxidation of plasma-derived fatty acids (24, 32) in DM2 subjects may lead to the accumulation of interstitial or cytosolic free fatty acids. Especially saturated fatty acids like palmitate can directly stimulate the production of IL-6 in myocytes from DM2 patients (13), when present in abundance. So, impairments in substrate handling, as found in IGT and DM2, may lead to the production of free radicals, and/or to an increment of local concentrations of saturated free fatty acids. Each of these may stimulate the production of IL-6 in the postprandial phase. The physiological significance of IL-6 release from muscle is still unclear. Pedersen *et al.* (35) suggested that IL-6 may be an interorgan signaling molecule during exercise, reporting a signal from skeletal muscle to the liver to indicate the use of glycogen. However, this is not likely to be the case after meal intake.

What may be the effect of the cytokines that are produced on skeletal muscle metabolism? It has been shown that systemic IL-6 infusion, resulting in high arterial IL-6 concentrations (~ 150 pg/ml), stimulates lipolysis and fatty acid oxidation in humans in rest (36), although it is not known whether similar effects will be found in the physiological range. However, in adipose tissue, it has been shown that interstitial IL-6 concentrations may be up to approximately 100 times as high as plasma values (37). Likewise, high interstitial amounts of IL-6 may affect fatty acid metabolism in an auto- or paracrine manner. On the other hand, it has been reported that IL-6 infusion did not affect glucose uptake or oxidation, and *in vitro* and *in vivo* studies show no effect of IL-6 on insulin action in skeletal muscle (12–14). However, IL-6 may induce tissue insulin resistance in other organs, *e.g.* in liver (11, 12), thus contributing to the further progression of IGT to DM2.

After weight loss, arterial IL-6 concentrations had not changed, but the postprandial release of IL-6 from muscle was decreased (-52% , $P = 0.04$). This improvement in IL-6 release after weight loss may have been a consequence of the reduction in body weight, the improvement of glucose tolerance and/or the increase in insulin sensitivity. This supports the concept that the postprandial increase in IL-6 pro-

duction may have been triggered by disturbances in glucose and/or fat metabolism found in obese IGT men.

The lack of differences between NGT and IGT subjects in arterial concentrations of IL-6, CRP, TNF-R1, and TNF-R2 is in accordance with some (5, 38), but not all studies (3) and may be explained by the fact that the observed groups are equally obese and only slightly different in insulin resistance.

In conclusion, we observed IL-6 release from forearm muscle in obese NGT and obese IGT subjects, both during fasting and after a meal. IL-6 release was significantly higher in IGT men during the postprandial phase when compared with obese NGT men. In these IGT men, the postprandial IL-6 release decreased after body weight loss, reaching levels that were found in the obese, NGT controls. We suggest that the high-fat meal may be an important metabolic stressor, evoking IL-6 release from skeletal muscle, due to the impairment in substrate handling in IGT men and that the IL-6 production may rather be a consequence than a cause of the obese, insulin-resistant and/or IGT state. Further studies have to provide more information on the mechanisms that are responsible for the increased IL-6 production by muscle, and whether the production of IL-6 in muscle may lead to a further increase in insulin resistance, contributing to the progression of IGT to DM2.

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References

1. Hanley AJ, Festa A, D'Agostino Jr RB, Wagenknecht LE, Savage PJ, Tracy RP, Saad MF, Haffner SM 2004 Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. *Diabetes* 53:1773–1781
2. Laaksonen DE, Niskanen L, Nyyssönen K, Punnonen K, Tuomainen TP, Valkonen VP, Salonen R, Salonen JT 2004 C-reactive protein and the development of the metabolic syndrome and diabetes in middle-aged men. *Diabetologia* 47:1403–1410
3. Muller S, Martin S, Koenig W, Hanifi-Moghaddam P, Rathmann W, Haastert B, Giani G, Illig T, Thorand B, Kolb H 2002 Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF- α or its receptors. *Diabetologia* 45: 805–812
4. Fernandez-Real JM, Broch M, Ricart W, Casamitjana R, Gutierrez C, Ven-

- drell J, Richart C 1998 Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. *Diabetes* 47:1757–1762
5. Dzienis-Straczewska S, Straczewski M, Szelachowska M, Stepień A, Kowalska I, Kinalska I 2003 Soluble tumor necrosis factor- α receptors in young obese subjects with normal and impaired glucose tolerance. *Diabetes Care* 26:875–880
 6. Trikha M, Corringham R, Klein B, Rossi JF 2003 Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. *Clin Cancer Res* 9:4653–4665
 7. Krogh-Madsen R, Møller K, Dela F, Kronborg G, Jauffred S, Pedersen BK 2004 Effect of hyperglycemia and hyperinsulinemia on the response of IL-6, TNF- α , and FFAs to low-dose endotoxemia in humans. *Am J Physiol Endocrinol Metab* 286:E766–E772
 8. Bennermo M, Held C, Stemme S, Ericsson CG, Silveira A, Green F, Tornvall P 2004 Genetic predisposition of the interleukin-6 response to inflammation: implications for a variety of major diseases? *Clin Chem* 50:2136–2140
 9. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM 2001 C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327–334
 10. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF 2003 Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52:812–817
 11. Senn JJ, Klover PJ, Nowak IA, Mooney RA 2002 Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 51:3391–3399
 12. Klover PJ, Zimmers TA, Koniaris LG, Mooney RA 2003 Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* 52:2784–2789
 13. Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Haring HU, Schleicher ED 2004 Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor- κ B. *J Biol Chem* 279:23942–23952
 14. Røtter S, Sørensen V, Larsson BM, Johansson A, Holmang A, Smith U 2004 Short-term infusion of interleukin-6 does not induce insulin resistance in vivo or impair insulin signalling in rats. *Diabetologia* 47:1879–1887
 15. Steensberg A, Fischer CP, Sacchetti M, Keller C, Osada T, Schjerling P, van Hall G, Febbraio MA, Pedersen BK 2003 Acute interleukin-6 administration does not impair muscle glucose uptake or whole-body glucose disposal in healthy humans. *J Physiol* 548:631–638
 16. Orban Z, Remaley AT, Sampson M, Trajanoski Z, Chrousos GP 1999 The differential effect of food intake and β -adrenergic stimulation on adipose-derived hormones and cytokines in man. *J Clin Endocrinol Metab* 84:2126–2133
 17. Keller P, Keller C, Carey AL, Jauffred S, Fischer CP, Steensberg A, Pedersen BK 2003 Interleukin-6 production by contracting human skeletal muscle: autocrine regulation by IL-6. *Biochem Biophys Res Commun* 310:550–554
 18. Fischer CP, Hiscock NJ, Penkowa M, Basu S, Vessby B, Kallner A, Sjøberg LB, Pedersen BK 2004 Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. *J Physiol* 558:633–645
 19. Febbraio MA, Steensberg A, Starkie RL, McConnell GK, Kingwell BA 2003 Skeletal muscle interleukin-6 and tumor necrosis factor- α release in healthy subjects and patients with type 2 diabetes at rest and during exercise. *Metabolism* 52:939–944
 20. Jellema A, Plat J, Mensink RP 2004 Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. *Eur J Clin Invest* 34:766–773
 21. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari A, Paolisso G, Marfella R, Giugliano D 2002 Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 39:1145–1150
 22. Siri W 1956 The gross composition of the body. *Adv Bio Med Physiol* 4:239–280
 23. Wallace TM, Matthews DR 2002 The assessment of insulin resistance in man. *Diabet Med* 19:527–534
 24. Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ, Heidendal GA, Saris WH 2000 Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab* 279:E146–E154
 25. Elia M, Folmer P, Schlattmann A, Goren A, Austin S 1988 Carbohydrate, fat, and protein metabolism in muscle and in the whole body after mixed meal ingestion. *Metabolism* 37:542–551
 26. Castell JV, Geiger T, Gross V, Andus T, Walter E, Hirano T, Kishimoto T, Heinrich PC 1988 Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat. *Eur J Biochem* 177:357–361
 27. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Copeck SW 1997 Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *J Clin Endocrinol Metab* 82:4196–4200
 28. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW 2004 Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 145:2273–2282
 29. Penkowa M, Keller C, Keller P, Jauffred S, Pedersen BK 2003 Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *FASEB J* 17:2166–2168
 30. Kosmidou I, Vassilakopoulos T, Xagorari A, Zakynthinos S, Papapetropoulos A, Roussos C 2002 Production of interleukin-6 by skeletal myotubes: role of reactive oxygen species. *Am J Respir Cell Mol Biol* 26:587–593
 31. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Picconi L, Bais B, Da Ros R, Motz E 2002 Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 106:1211–1218
 32. Mensink M, Blaak EE, van Baak MA, Wagenmakers AJ, Saris WH 2001 Plasma free fatty acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. *Diabetes* 50:2548–2554
 33. Tsai WC, Li YH, Lin CC, Chao TH, Chen JH 2004 Effects of oxidative stress on endothelial function after a high-fat meal. *Clin Sci (Lond)* 106:315–319
 34. Ceriello A, Bortolotti N, Motz E, Crescentini A, Lizzio S, Russo A, Tonutti L, Taboga C 1998 Meal-generated oxidative stress in type 2 diabetic patients. *Diabetes Care* 21:1529–1533
 35. Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, van Hall G, Plomgaard P, Febbraio MA 2003 Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. *Pflugers Arch* 446:9–16
 36. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Møller K, Saltin B, Febbraio MA, Pedersen BK 2003 Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab* 88:3005–3010
 37. Sørensen VR, Sandqvist M, Gustafson B, Hammarstedt A, Schmelz M, Yang X, Jansson PA, Smith U 2004 High local concentrations and effects on differentiation implicate interleukin-6 as a paracrine regulator. *Obes Res* 12:454–460
 38. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B 2000 Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* 85:3338–3342

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